TSE
Technical & Scientific
Equipment GmbH

TSE Fear Conditioning System

for small laboratory animals

T S E Fear Conditioning System

1. Introduction

1.1. The system

The new TSE **Fear Conditioning System** (FCS) has been developed to study conditioned fear reflexes in small laboratory animals (mice or rats). Shock induced fear is usually expressed as reduced activity, especially freezing behaviour: the animals tend to remain in motionless, defensive posture. The FCS allows a complex analysis of different aspects of this behaviour and can therefore be used to examine the psychopharmacology of fear conditioning and to investigate into the effects of anxiolytic drugs.

The FCS system consists of the following components:

- Up to 4 **boxes** with animal location sensors and shockable grid
- Box **housing** with loud speaker, light and ventilator
- **Control unit** with integrated shocker/scrambler
- FCS-**Software**
- FCS-**Interface** to be built into an IBM-compatible computer

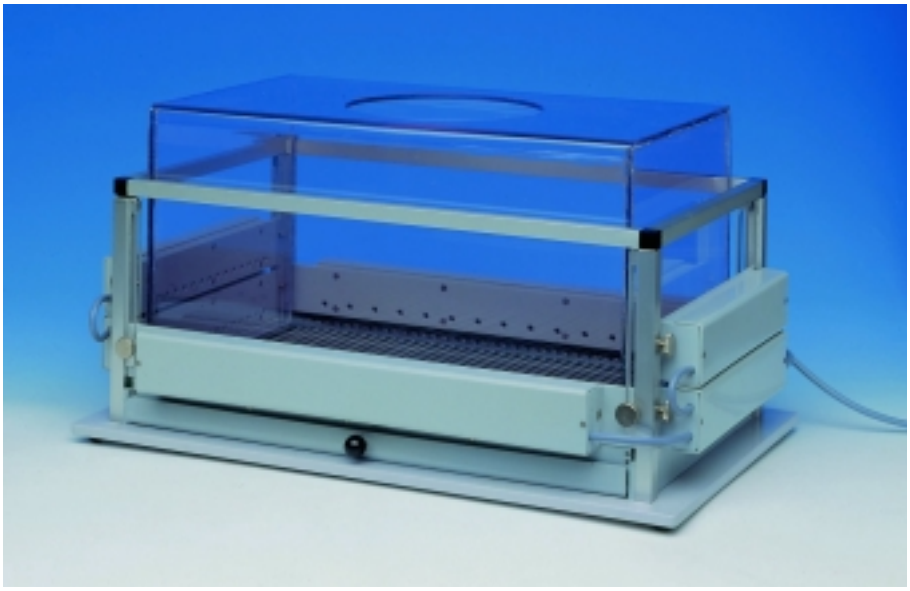
1.2. Components

The specially constructed test box comprises:

- Rectangular **base construction** with sensor electronics and frame with integrated animal detection **sensors**.
- Removable stainless steel **foot shock grid**.
- **Arena** with clear acrylic lid (with central cutout).
- **Separator plate** (clear acrylic) to be placed diagonally into the arena in order to restrict the test field (modification of environmental conditions).
- Removable **feces tray**.

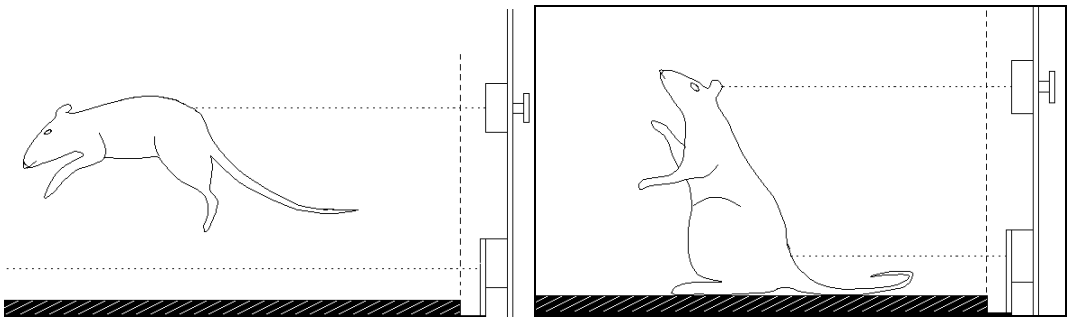
The box dimensions will be manufactured according to customer specification.

Example:	**Mouse**	350 x 200 x 200 mm (wxdxh of arena) Grid rods 4mm ∅ mounted 0.9cm apart



The animal´s position and movement inside the cage is monitored with the help of infra-red light barriers, scanned continuously with a frequency of 10 Hz (105ms). The sensors are mounted in 2 planes: the **first** sensor level (2x15 sensors) is mounted approx. 13mm above the floor grid and is used to monitor the horizontal coordinates of the animal (x- and y-direction).

With the help of the height-adjustable **second** sensor level Z (15 sensors) variable between 23mm and 80mm above the floor grid) rearings and jumps can be detected.



Rearing:	Both sensor levels are interrupted
Jump:	Only second level detects the animal

The box is contained in a sound attenuating housing featuring:

- hinged front door with integrated inspection window,
- loud speaker (sound or white noise) and light in ceiling construction,
- ventilator in side wall (constant speed),
- sliding floor plate for easy removal of test box.

The housing can be equipped with a red LED that can be operated via the software. This diode is used to signal a time window where user-defined behaviours of the animal are supposed to be recorded manually.

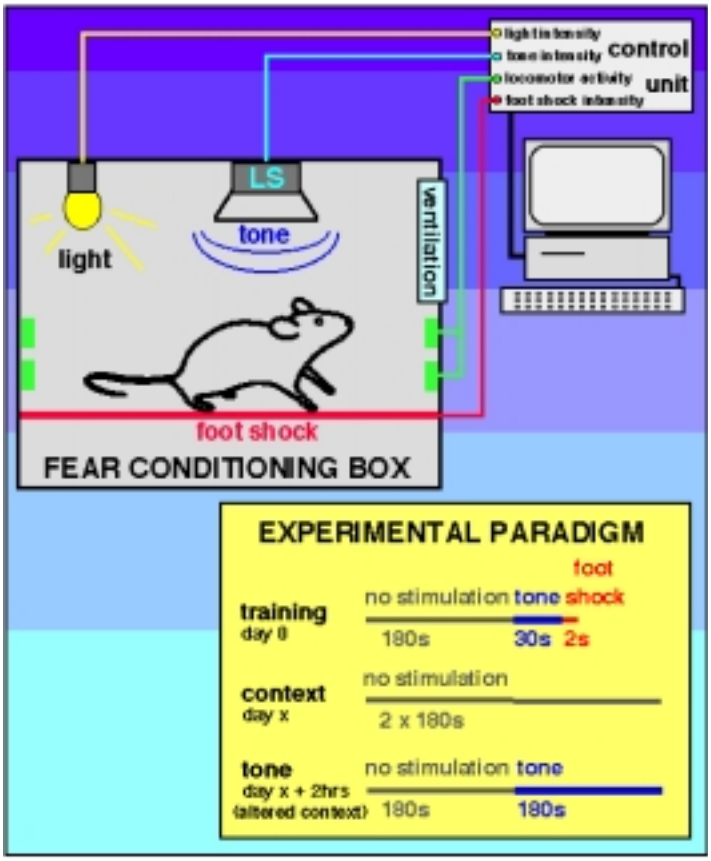
The control unit - in its standard version configured for parallel control of up to 4 fear conditioning boxes - features:

- **sound generator** for generating an auditory (conditioned) stimulus (Sinus 10kHz for mice, 1kHz for rats), clicking with variable, user-defined frequency (in ms); switched ON/OFF via software,
- **noise generator** to provide background white noise; switched ON/OFF via software
- **light source** for generating a light (conditioned) stimulus; switched ON/OFF via software,
- **amplifier** for auditory (conditioned) stimulus and noise signal (output max. 100 dB),
- sound/noise amplitude (volume) and light intensity adjustment **knobs**,
- **shocker/scrambler** *(one shocker per box required)*: microprocessor controlled constant current generator with integrated current flow detector for pole reversal of grid rods. Software-adjustable amplitude (standard configuration: max 3.1mA; up to 4.5mA on request; adjustable in steps of 0.1mA) and duration. Alternatively the user can choose pulsating stimulus current (standard frequency: 20Hz, other frequencies available on request) via software.

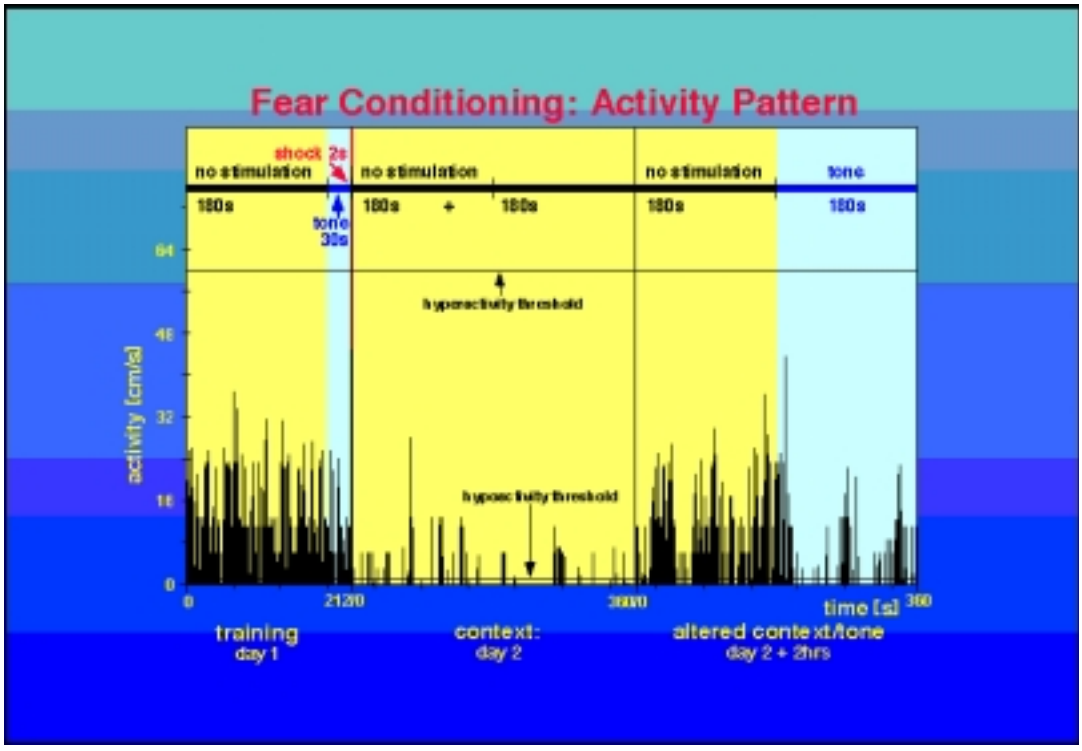
The FCS software package is always used in conjunction with the FCS interface. This interface is designed to be built into an IBM-compatible computer. We can supply you with a complete system including the computer completely installed and ready for use! The software controls the test in the boxes, collects, displays and stores all experimental data (animal movement data) and allows for detailed analysis and documentation. The software runs under the operating system Windows.

1.3. The fear conditioning paradigm

In the **fear conditioning paradigm** the subject is given a auditory cue (conditioned stimulus) followed by a short electric shock or only an electric shock (unconditioned stimulus). This training is called the conditioning trial.



Schematic representation of the fear conditioning paradigm



Animal Activity - Graphical Evaluation

The animals can subsequently be tested for their fear to both the context and to the auditory cue. Normal adult rats usually show a conditioned fear response expressed as reduced activity resp. freezing to both the context and to the sound. This retention test can be conducted immediately after the training or some days later (variation of the length of the retention interval) and can thus give information about short-term and long-term memory influences on conditioning.

In the following example the test consists of 3 phases:

Phase 1	*Acquisition*	No stimulus	180s
		Sound (CS)	30s
		Shock (US)	2s
		No stimulus	20s
Phase 2	*Context*	No stimulus	180s
		No stimulus	180s
Phase 3	*Sound*	No stimulus	180s
	(New context)	Sound (CS)	180s
		No stimulus	60s

Phase 1 respresents the training trial, where the animal is conditioned to the sound stimulus. Phase 2 is conducted with the animal placed into the original conditioning context to record contextual fear conditioning, i.e. freezing, whereas in phase 3 the context is altered by placing the separator plate into the box thus changing the area from a rectangle into a triangle in order to assess auditory fear conditioning.

The sequence of stimulus presentation is determined in an ASCII control file written by the operator using several simple commands. Up to 10 test phases can be defined each consisting of up to 5 sub-phases. In addition the control file defines:

- intensity (mA)/duration of electric shock, type of current (constant/pulsating),
- duration and click frequency of the sound (duration sinus=duration noise),
- whether the light is to be used as stimulus or house light.

In the absence of the sound the loud speaker continuously produces a background noise signal. During the sound the noise signal is switched off.

The program design supports three consecutive steps:

- Test preparation
- Test run
- Data analysis

After starting the software the user first defines the test phase, activates the boxes and enters animal and experimental identifiers.

During the experiment the operator is provided with detailed status information for all boxes, e.g. current position of the animal, number of moves, active stimuli. The system automatically records all animal position coordinates for subsequent analysis.

After the experiment has been finished the measured data are stored on the hard disk together with all animal and experimental identifiers. A list of raw data is available showing all recorded position coordinates. These data can now be analysed using the integrated analysis algorithms.

The figure clearly shows the animal´s contextual fear during phase 2 of the experiment, where no stimulus is given. The same animal doesn´t show any freezing reaction after altering the context (phase 3, part 1). In the auditory cue test (phase 3, part 2) an obvious fear conditioning reaction can be observed.

All numerical data can be saved in export files for further analysis. To date 3 different formats are available to guarantee compatibility with most commercial spread sheet programs or statistical packages.

2. Experimental procedure

2.1. Experiment structure

The structure of the experiment is defined via the control file **AKS.CON**, a simple ASCII file, which can be edited by the user with the help of any editing program.

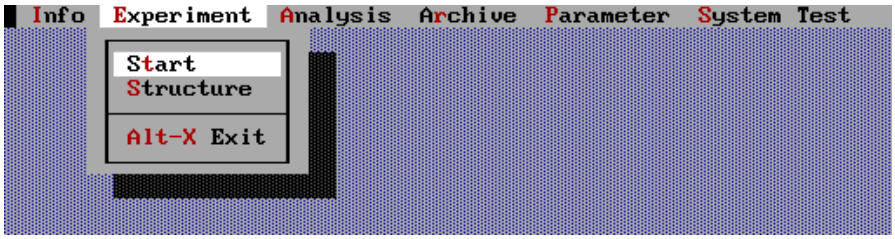
```
Control File  FCS-System

@PHASE 1
PAUSE
30
SOUND
30
ESTIM
30
0.7
PULS
PAUSE
30
;
@PHASE 2
PAUSE
30
;
@PHASE 3
PAUSE
60
SOUND
40
PAUSE
40
;
@SOUNDDELAY
100
;
@LIGHTSTIMULUS
0
```

All inputs have to be made according to the following definitions:

- Each phase starts with the header @PHASE.
- The sub-phases are defined with SOUND, PAUSE, LIGHT, ESTIM and their duration in seconds
- The electrical shock is additionally specified by entering the intensity in mA and the type of current (CONSTANT oder PULS).
- SOUNDDELAY specifies the click frequency of the sound signal in milliseconds (the exact value is a multiple of 7ms)
- The light can be used as house light (LIGHTSTIMULUS = 0) or as stimulus light (LIGHTSTIMULUS = 1).

  House light will be turned on with pressing the test start button and will burn throughout the whole experiment.

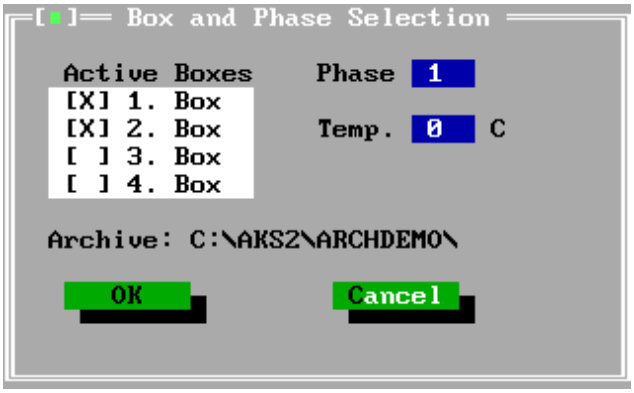
2.2. Test preparation



After starting the FCS program the menu item ***Experiment/Structure*** allows the user to check whether the entries in the control file are interpreted correctly by the system.

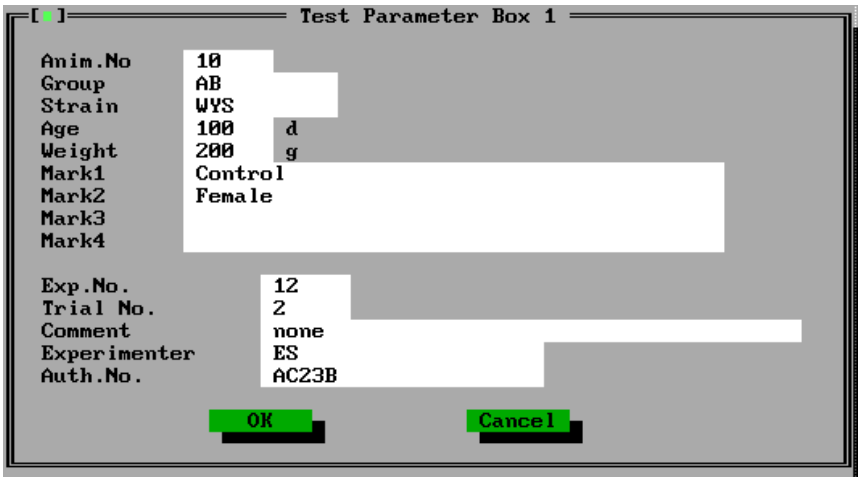
```
Test Control FEAR CONDITIONING SYSTEM

Phase No. 1
       Pause  30  s
       Sound  30  s
       E-Stim 30  s    0.7 mA  puls
       Pause  30  s
Phase No. 2
       Pause  30  s
Phase No. 3
       Pause  60  s
       Sound  40  s
       Pause  40  s

Sound    100  ms
Light =  House Light
```

If the structure of the data file is correct the ***Experiment/Start*** menu item is activated. The user now chooses the number of boxes to be integrated into the test and enters the desired phase (1..10). This entry will define the following program step.



2.3. A new experiment

Entering **Phase 1** will start a new experiment, entering a number between **2** and **10** will start a follow-up trial. In **Phase 1** the operator is asked to enter all animal and experimental identifiers for the selected boxes. These entries are valid for all phases and any subsequent changes are prevented by the system!



Test Parameter

Animal Identifiers
- Animal No.
- Group
- Strain
- Age
- Weight
- Mark 1..4

Experiment Identifiers
- Experiment No.
- Trial No.
- Comment
- Experimenter
- Authorization No.

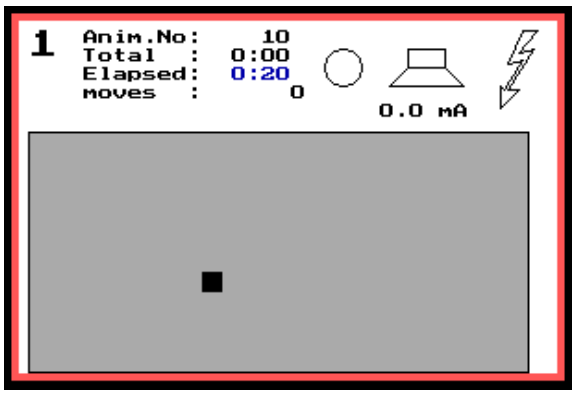
The animal number is reflected in the data file name: all data of one animal will be saved to one file named **AKxxxxxx.DAT** with **xxxxxx** representing the animal number.

2.4. Follow-up trial

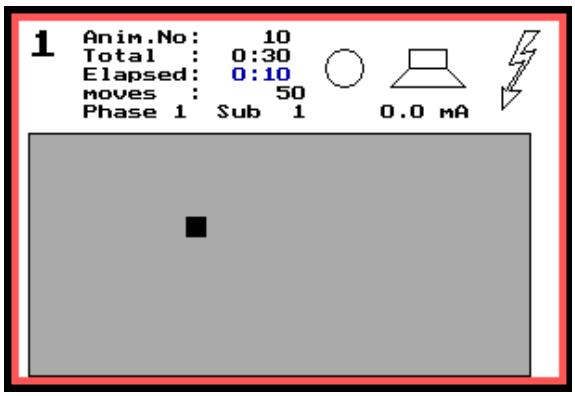
Entering a phase number >1 will open a window where all data sets available for a follow-up trial with this phase number are listed for selection. The following test data will be added to the already existing data file.

2.5. Test start

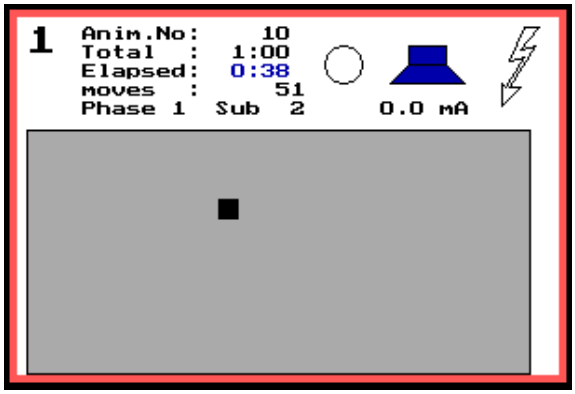
After all entries have been made in the „test parameter“ window or after up to 4 already existing animals have been selected for a follow-up trial the so-called ***test monitor*** will be displayed, showing all 4 boxes simultaneously on the computer screen. House light (if not used as stimulus) as well as noise are switched on. Now the animals have to be placed into the appropriate boxes.



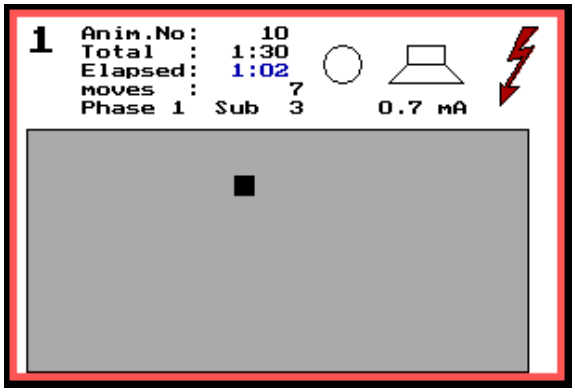
....before test start



.....after pressing the start button



Sound Cue



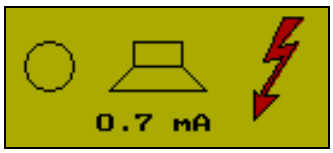
Electric Shock

Pressing a single key will then start the test in all boxes simultaneously.

During the trial the animals´ positions inside the boxes are continuously displayed on the computer screen (square) together with the following status information:

- total time to end of current subphase
- time already elapsed after pressing the start button
- the number of moves (interruptions of light barriers)
- the current phase and subphase number

Graphical symbols represent the active stimuli:



- yellow circle — active light
- blue loud speaker — active sound
- red flash (+ intensity in mA) — active E-stimulus

2.6. Manual recording of behaviour

During data acquisition the user can enter event markers into the data file using the keyboard. This function serves as an event recorder and can be allocated according to customer´s specification to any behavioral event to be registered additionally such as grooming, freezing, rearing an so on. The LED mounted above the inspection window can be lighted in a user-defined sequence in order to remind the operator to do so. The markers are later output in a table.

Note: The system automatically recognizes freezing behaviour during analysis according to the parameter „freezing“ defined in the menu point ***analysis parameter***.

2.7. Test end and data storage

All data are stored on the hard disk at regular intervals during the test. All parameters and data for *one* animal are stored in *one* file. The operator can determine a directory in which the test data are to be stored, the so-called data directory. This structuring of data during recording avoids a confusing mass of data on the hard disk.

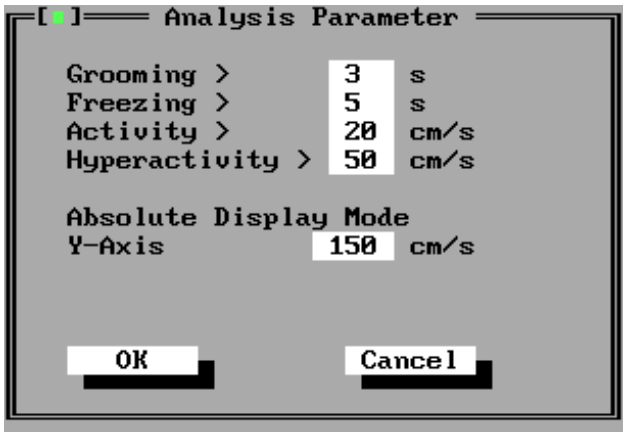
When all sub-phases of the chosen phase have been executed a test is ended automatically in all boxes simultaneously. Alternatively the user can terminate the test manually. The FCS system prevents the program being terminated inadvertently when boxes are still active. This procedure is in accordance with the provisions of the Good Laboratory Practice code (GLP).

3. Evaluation

The analysis of stored test data is carried out in two stages. In the ***first*** stage the data to be used for analysis are selected by data selection. The data sets can be preselected with the aid of a filter, in which parameters defined during test preparation are used for filtering criteria; this makes the handling of even large amounts of data much easier. These selected data sets are then subjected to different analysis algorithms.

3.1. Analysis parameters

The analysis of the measured data is modified by the so-called „analysis parameter“ which have to be defined beforehand.



Grooming (in seconds)
Time threshold for grooming behaviour. Grooming is interpreted whenever a rearing (=sensor level Z interrupted) has exceeded this duration.

Freezing (in seconds)
Time threshold for freezing behaviour. Freezing is interpreted whenever the animal hasn´t been moving for more than this duration.

Activity (in cm/s)
Speed threshold for „activity“. The threshold will be seen in the histogram as a horizontal line.

Hyperactivity (in cm/s)
Speed threshold for „hyperactivity“. The threshold will be seen in the histogram as a horizontal line.

In case the animal´s speed exceeds the **activity** resp. the **hyperactivity threshold** (cm/sec) an internal counter is incremented. Simultaneously the system registers the duration of the activity/hyperactivity behaviour.

3.2. Raw data

With the menu item ***Raw Data*** the animal´s position coordinates are listed in detail in a table. At the beginning all the information can be found which characterizes the data set. The table, which can also be printed, has the following columns:

- The first three columns represent the X- resp. Y-coordinate as well as the Z-value in the intrasystemic data format.
- Column 4 represents the time in ms beginning from the start.

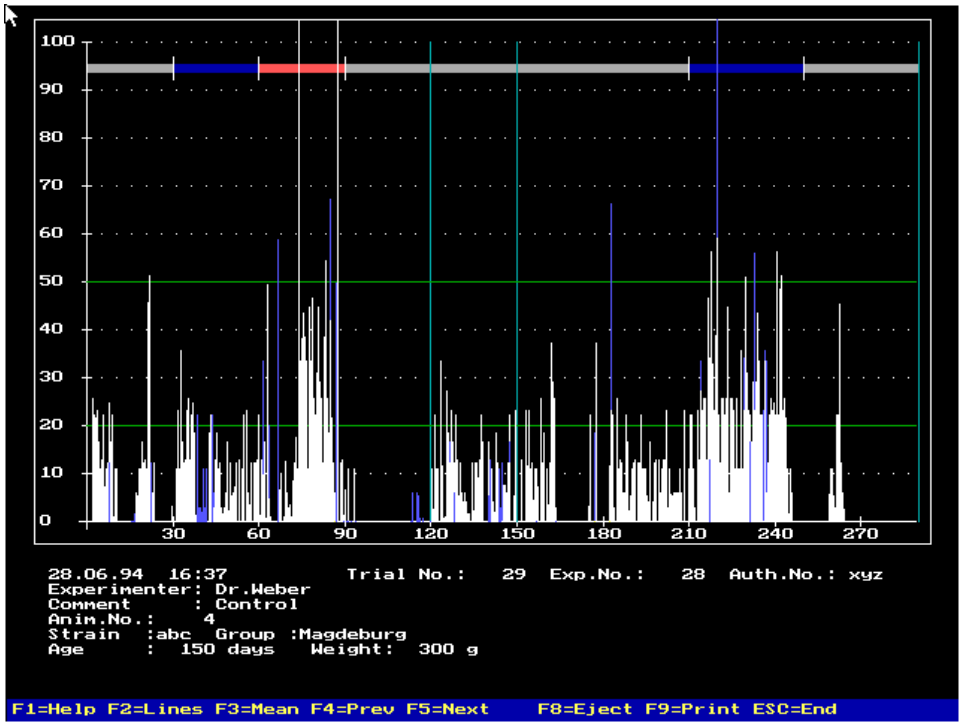
- The next 2 columns represent the X- resp. Y-coordinate converted into cm.
- The last column is reserved for events such as rearings, jumps and manually entered events.

```
         Raw Data  FEAR CONDITIONING SYSTEM


         Anim.No.:   4     Trial No.:   1     Exp.-No.:   1
         Control
         Phases: 3
         Activity > 1  cm/s       Grooming > 3 s
         Hyperact.> 60 cm/s       Freezing > 5 s

         Phase: 1       28.06.95     14.00
             Pause  30s   38 Values
         IndA=  1     IndE=  38      Qty=  38
           145   15    0          0ms       31.8cm    0.7cm
           140   15    0       7322ms       30.6cm    0.7cm
           140   20    0       7427ms       30.6cm    1.3cm
           135   20    0       7637ms       29.5cm    1.3cm
           135   15    0       8267ms       29.5cm    0.7cm
           125   15    0       8372ms       27.1cm    0.7cm
           115   15    1       8687ms       24.7cm    0.7cm      Rearing
           105   15    0       8897ms       22.4cm    0.7cm
            95   15    0       9002ms       20.0cm    0.7cm
            85   15    0       9107ms       17.7cm    0.7cm
            75   15    0       9317ms       15.3cm    0.7cm
            65   20    0       9422ms       13.0cm    1.3cm
            55   20    2       9527ms       10.6cm    1.3cm      Jump
            55   15    0       9632ms       10.6cm    0.7cm
            45   15    0       9737ms        8.2cm    0.7cm
etc

         Sound  30s        24 Values
         IndA=  29    IndE=  62      Qty=  24
           20   145   0      33677ms        2.4cm    17.6cm
           25   145   0      33782ms        3.5cm    17.6cm
           15   145   0      33887ms        1.2cm    17.6cm

etc
```

3.3. Histogram

The graphic supplies information about the animal´s pattern of activity during the course of the experiment. Each selected data record, i.e. one experiment with one animal, is displayed in a separate graphic.

Measure of activity is the animal´s momentary speed (cm/sec). This speed is displayed as a vertical line in a coordinate system with the **time** (in seconds) as the X-axis and the **speed** (in cm/sec) as the Y-axis together with the chosen thresholds for activity and hyperactivity (horizontal lines).



The colour of the speed line has the following meaning:

white	Detection in horizontal plane
blue	Rearing (detection in x,y and z)
yellow	Jump (detection only in z)
green	manually recorded event

The different phases of the experiment are separated by vertical lines. A bar at the upper screen displays the different subphases of each phase with a colour corresponding to the „colour“ of the stimulus used:

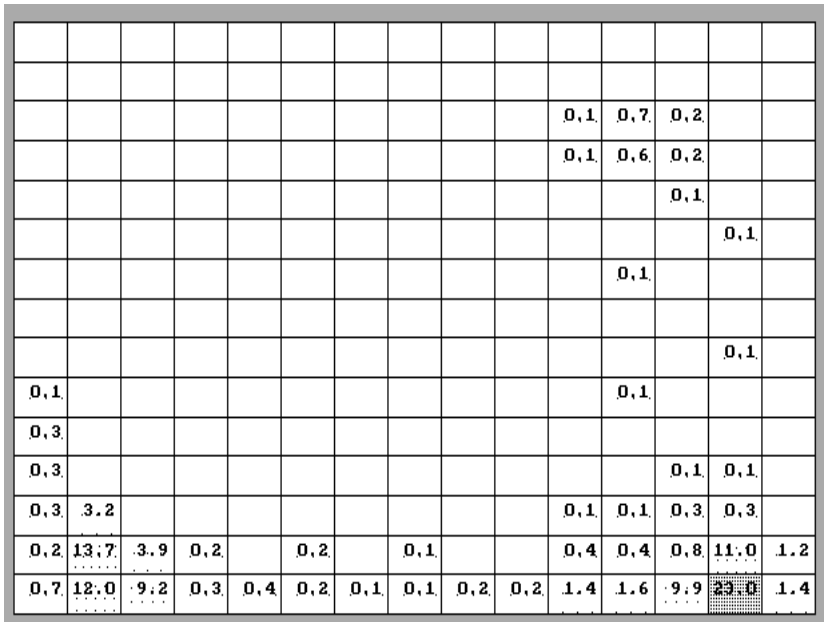
grey	Pause
blue	Sound
yellow	Light
red	E-Stimulus

The display can be switched to show the mean speed for each sub-phase.

3.4. Spacial analysis

The spacial analysis generates a graph and a table.

The **graph** provides an overview about the time distribution of the animal´s movement in the box. For each phase a separate graph is generated. The analysis is performed using the coordinates of the X- and Y- sensor levels.



The total box area is divided into 225 elements (15x15 light beams). For each element the system now calculates the total visit time of the animal. The percentage of visit time compared to the total time in the box is then output in each element. If the animal did not visit a specific area, the area in the graph is empty.

The elements are shaded with a pattern of dots in order to allow a quick overview about the animal´s length of stay.

The corresponding table lists the calculated percentages of visit time for each element in form of a grid. The values are arranged in 15 columns (x-coordinate) and 15 lines (y-coordinate). The sum of all values is 100%.

This table can be exported as ASCII file for further statistical calculations.

0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.7	0.2	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.2	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
0.3	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.3	0.3	0.0
0.2	13.7	3.9	0.2	0.0	0.2	0.0	0.1	0.0	0.0	0.4	0.4	0.8	11.0	1.2
0.7	12.0	9.2	0.3	0.4	0.2	0.1	0.1	0.2	0.2	1.4	1.6	9.9	23.0	1.4

3.5. Event marker table

The marker table lists the manually recorded behaviours with their frequency, total duration and latency for each phase and subphase.

Table of Results FEAR CONDITIONING SYSTEM

Ph	Type	Marker 1		Marker 2		Marker 3		Marker 4		Marker 5	
		No.	Dur.	No.	Dur.	No.	Dur.	No.	Dur.	No.	Dur.
1	Pause	1	1.0	2	10.0	0	0.0	0	0.0	0	0.0
	Sound	1	2.5	1	5.0	0	0.0	0	0.0	0	0.0
	E-Sti	1	3.5	0	0.0	0	0.0	0	0.0	0	0.0
	Pause	2	5.0	1	2.0	0	0.0	0	0.0	0	0.0
2	Pause	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Ph	Type	Marker 1 Latency	Marker 2 Latency	Marker 3 Latency	Marker 4 Latency	Marker 5 Latency
1	Pause	1.0	2.0	0.0	0.0	0.0
	Sound	2.0	1.0	0.0	0.0	0.0
	E-Sti	0.5	0.0	0.0	0.0	0.0

Etc.

3.6. Table of results

The ***table of results*** lists a series of behavioral parameters calculated from the raw data in detail. At the beginning of each table all animal and experimental identifiers as well as the analysis parameters are given.

Ph	Phase number
Type	Type of sub-phase (Pause, Sound, Light, E-Stim)
Dis(cm)	Distance travelled (cm)
Rear	Number of rearings
Rear %	Percent rearing time (referred to total sub-phase duration)
Gro	Number of grooming events
FrM	Manually entered actions (e.g. freezing events)
Fre	Number of freezing events

Jum	Number of jumps
Act	Actions exceeding the activity threshold
Act %	Percent activity time (referred to total sub-phase duration)
Hyp	Actions exceeding the hyperactivity threshold
Hyp %	Percent hyperactivity time (referred to total sub-phase duration)
VMean	Mean speed (cm/s)
VMax	Maximum speed (cm/s)
VSD	Standard deviation
VSEM	Standard error of arithmetic mean (SEM)
Rest	Resting time
Rest%	Percent resting time (referred to total sub-phase duration)

All these parameters can be used for further-reaching complex statistics in the form of export files. Currently the following export formats are supported: ASCII (suitable for e.g. EXCEL) and the dBase compatible SDF and CSV formats.

Table of Results FEAR CONDITIONING SYSTEM

Anim.No.: 4 Trial No.: 1 Exp.No.: 1
Control
Phases: 3
Activity > 1 cm/s Grooming > 1 s
Hyperact.> 60 cm/s Freezing > 1 s

PH	Type	Dis(cm)	Rear	%	Gro	FrM	Fre	Jum	Act	%	Hyp	%
1	Pause	929.0	10	3.7	2	0	10	0	38	83.9	2	0.0
	Sound	113.5	0	0.0	0	0	2	0	7	67.6	0	0.0
	E-Sti	40.3	1	5.2	0	0	1	0	1	98.3	0	0.0
2	Pause	116.8	1	0.5	0	0	2	0	18	13.1	0	0.0
	Pause	42.9	0	0.0	0	0	1	0	14	5.5	0	0.0
3	Pause	674.9	5	3.2	3	0	4	0	39	59.3	0	0.0
	Sound	279.3	2	0.2	0	0	1	0	33	24.9	0	0.0

PH	Type	Dis(cm)	Vmean	VMax	VSD	VSEM	Rest	%
1	Pause	929.0	5.10	168.4	6.62	0.16	96.1	53.39
	Sound	113.5	3.75	25.7	5.88	0.35	19.4	64.64
	E-Sti	40.3	20.14	45.3	13.93	3.19	0.2	10.84
2	Pause	116.8	0.64	28.1	2.55	0.06	167.5	93.06
	Pause	42.9	0.24	11.2	1.24	0.03	173.4	96.32
3	Pause	674.9	3.73	36.4	5.90	0.14	115.1	63.95
	Sound	279.3	1.55	43.6	4.13	0.10	152.2	84.54

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